THE FATE OF OXYTETRACYCLINE IN THE MARINE ENVIRONMENT OF A SALMON CAGE FARM.

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ABSTRACT

This paper gives a summary of previously published results of studies on the dispersal of oxytetracycline from the vicinity of a typical salmon farm. These studies showed the environmental impact of occasional treatments to be negligible.

Concentrations of oxytetracycline (OTC) were measured in the benthic sediments and in mussel *Mytilus edulis* sampled in the vicinity of an inshore salmon farm on the west coast of Ireland. Concentrations between 1.0 µg/g and 14.7 µg/g were observed in sediments within 120 m from the farm. Concentrations declined exponentially with time, reaching low levels after 32 days and reduced to traces at 66 days. The highest concentrations were observed in the top 2 cm of sediment, falling to trace levels at a depth of 10 cm. The half-life of OTC persistence in mussels was found to be approximately 2 days.

Residues in unpolluted sediment beneath the cages were never present in high concentrations and were flushed out rapidly. In the presence of excessive quantities of unconsumed food pellets on the seabed and in anoxic sediment, the persistence of OTC was significantly prolonged. Monitoring the quality of the sediment could therefore provide adequate indication of any risk of accumulation of antibiotic, without the need for elaborate chemical analyses.

Residues in sediment, invertebrates and salmon could account for not more than 1.3% of total input of OTC. It was concluded that the antibiotic was very rapidly dispersed in the environment and its use in salmon therapy posed no material risk to human or environmental health.

CONTENTS

INTRODUCTION

Chemotherapeutants have been widely and effectively used in marine salmon culture since the 1980's. Public concern as to the effects of antibiotic residues on human health and marine environment quality has led to stringent regulations on their use. The aim of this study was to determine the levels of antibiotic residues in the vicinity of a fish farm, in benthic sediments and in salmon and shellfish, which might eventually enter the human food chain. A model of the probable fate of OTC would be developed and the dominant factors influencing that fate identified. This account of the study, undertaken in the mid-1990s, gives a synthesis of the essential features of the results and conclusions, details of which have already been published (Coyne *et. al.*, 1994a, 1994b, 1996 and 1997). The salmon farming industry in 1997 contributed £37.5 million to the Irish economy by the production of over 15,000t of fresh salmon. This value is greatly increased by yielding such highly priced products as smoked salmon. Of equal importance is the fact that the majority of the rearing installations are located in remote coastal areas where year-round employment is scarce. Direct employment for the industry in 1997 was 1,051 (Anon. 1997a), with indirect employment being generated in many spin-off industries. Compared to other forms of food production, salmon farming has proven to be more economical, in that retention of dietary protein and energy is approximately double that of chicken and pig production and the waste is correspondingly lower.

Disease Control

Disease is the over-riding constraint within the aquaculture industry. At worst, fish mortality may extend to an entire season’s production. Even in cases where the fish recover, production may be interrupted for some time and will in all cases be affected by reduced growth.

The diversity of diseases requires a multifactorial approach to prevention and control. Good management practices are essential within the farm. Control of the movement of fish between farms needs to be rigorously enforced.

Vaccination has made an important contribution to disease control, but Austin and Austin (1993) caution that even the best vaccines do not completely prevent the occurrence of disease. Research by Hiney (1998) has shown that in covert infections (showing no clinical signs), vaccinated fish will, under artificial and natural stress conditions, precipitate clinical furunculosis. Moreover, vaccinated covertly infected fish have been shown to transmit the disease to fish that are free of furunculosis. These results perhaps serve to warn against over reliance on vaccines.

The data of Wheatley (1994) showed that the annual losses due to furunculosis in farms in Irish waters were approximately 1% during the period 1988-92. Smith (1992) considered that this low level of losses had been achieved by controlling the movement of covertly infected fish, in the absence of oil-based vaccination and in an industry that had experienced furunculosis in sea farms since 1978.

Antibiotic therapy represents the final line of defence. Availability of antimicrobial agents capable of controlling disease in farmed salmonids that are not protected by vaccination is therefore vital.

Control of chemical treatment

In the early 1980's, when the industry was beginning to be productive, farmers had a large variety of chemotherapeutants (antibacterial and anti-lice treatments) at their disposal. However, public concern developed over the use of such chemicals for two reasons: (1) they were being used in a novel environment and (2) antibacterial agents have a biological activity at very low concentrations. In the course of the next two decades, their use became subject to regulation.

The availability of chemotherapeutants for use in aquaculture has been severely restricted by the European Union, first in 1990 by Council Regulation 2377/90, then by Council Regulation 2309/93. Following this the European Medicines Evaluation Agency (EMEA) was formed in 1993. It is responsible for both human and veterinary medicine and the EU's Committee for Veterinary Medicinal Products (CVMP) is now a committee within its structure.

More than 45 items of EU legislation apply to the use of veterinary medicines in aquaculture. The majority relate to two main areas, marketing authorisation (licensing) and control of residues in edible tissues. The industry now is subject to national, European and international regulatory constraints.

Within the regulations, the following antimicrobial agents are available for use in aquaculture in Ireland:

- OTC: oxytetracycline (Tetraplex)
- flumequine
- amoxicillin (Aquacil)
- sulphadiazine potentiated with trimethoprim (Sulfatrim).

Directive 90/676/EEC (1990) establishes the prescribing cascade. This means that, where there is no suitable authorised product to treat fish, the veterinarian may use a suitable product approved in other food animal species. The use of this prescription route requires the imposition of a standard withdrawal period and, for fish in the EU, this has been set at 500-degree days (Alderman, 1999). Alderman explains that a significant restriction on the cascade system is that a product may still only be applied by the exact route for which it is authorised and only a “small number” of animals may be treated under the system.

One of the main problems in licensing veterinary medicines for aquaculture lies in establishing the maximum residue levels (MRL) allowed in target edible tissues of different species. Recently the EMEA has published a Note for Guidance (Anon. 1997b) where an extrapolation can be considered acceptable whereby, if an MRL has been established for a substance in the muscle of a major mammalian species, it may be applied to Salmonidae and to other finfish.

Chemotherapeutants in the environment

The use of chemical treatments in fish farms is an issue that has raised concerns in the minds of the general public and of the scientific community. In the 1980’s, little or no data was available on the possible consequences of the use of therapeutants in the marine environment. Thus, some concern was justified. However, in the 1990’s,

a significant amount of data was accumulated from field studies and laboratory experiments.

Lunestad (1992) outlined the distribution of an antibacterial agent in the marine environment as originating with oral administration of medicated feed to fish. The agent may be released from the pellets in dissolved form and, depending on the substance, may be chemically or microbiologically degraded, diluted in the water masses or absorbed by the fish and other marine organisms. The fate of fragments of feed pellets and faecal particles, containing the agent may be as follows; (1) dispersion by the marine currents affecting the farm site; (2) ingestion by wild fish and filter-feeding organisms such as mussels and oysters; (3) transportation to the sediment.

The fate of an agent associated with fish faeces will depend on the 'quality of the faeces'. O'Connor (1998) describes salmon faeces as a “puff of white liquid”. Marine currents, making sedimentation an unlikely event, would readily disperse this “puff”.

The majority of studies have concentrated on the fate of OTC in the environment of fish farms. This emphasis has resulted from its frequency of use in the industry in the 1980’s and its importance in both animal and human medicine. Data on the environmental fate of OTC, are available from over 17 farm sites in three countries: Norway (Jacobsen and Berglind, 1988, Samuelsen *et al*., 1992a), Finland (Bjørklund *et al*., 1990) and USA (Capone *et al.,* 1996). The primary aim in all these studies was to determine its concentration and persistence in the fish farm sediment. Sediment levels reported ranged from 0.1 µg/g to 285 µg/g and half-life in the sediment from 9 to 419 days. Traces of OTC were also found in sediments adjacent to the farm.

The majority of investigators, in designing their fieldwork, seem to have accepted that deposition on, or in, the sediment was the most important of the possible fates of OTC. However, Møster (1986), Samuelsen *et al*. (1992a) and Capone *et al*. (1996) measured concentrations in filter feeding organisms such as mussels and oysters, sampled in the vicinity of a farm.

Directive 81/851/EEC, as amended by 92/18/EEC, requires assessment of the potential environmental risks associated with the use of veterinary medicinal products. The Joint Group of Experts on the Scientific Aspect of Marine Environmental Protection (GESAMP), was established by a number of organisations within the United Nations (IMO, FAO, UNESCO-IOC, WHO, IAEA, UN, UNEP). It drew attention to the fact that very little is known about the environmental fate of many aquaculture drugs (Anon. 1997c). CVMP requires the manufacturer’s application for marketing authorisation for veterinary medicinal products to include a complete report, which would conclude with an environmental risk assessment (ERA), based on characteristics of the product, its potential environmental exposure and environmental fate (Anon. 1997b).

Knowledge of the fate of a chemical is an essential factor in investigating its effect in the environment. In due course, fate and effect data can be used to support

decisions for environmental management and risk assessment for marine waters (Suter II, 1993). Management schemes require the formulation of models of predictive environmental concentration (PEC). These require field data for validation.

The wide variations reported in the previous studies clearly indicate that local conditions, both of husbandry and environment, play a large part in determining the fate of the residues. The published results therefore could not be meaningfully applied to any situation in Ireland and comprehensive local studies would be essential. While comparable data on the persistence of OTC in target species and in sediments adjacent to fish farms were widely available, information on other aspects was more limited.

The essential question was whether OTC residues, derived from Irish fish farms, persist to a degree likely to cause any risk to human health or marine environment quality. Logistical considerations demanded that the work be carried out at a single salmon farm. Previous studies had shown clearly that detectable quantities of residues could be traced in sediments and in sedentary molluscs and these were therefore selected as the main targets. The results should indicate whether there is any cause for concern as a result of current disease treatment practice in fish farms and provide a baseline for future studies and monitoring.

The investigation was carried out in four separate studies at the same fish farm with the following aims:

- *Study 1* to measure the horizontal and vertical distribution of the residues resulting from therapy in a single cage block and the half-life of persistence in the sediment.
- *Study 2* to compare levels resulting from treatment in both blocks of cages but at different dosages.
- *Study 3* to establish the concentration and half-life of OTC in sediment, the mussel *Mytilus edulis* and the salmon at cage Block 6.
- *Study 4* to determine concentration and half-life of OTC in sediment, salmon muscle and mussel tissue after prolonged treatment. Attempts would also be made to sample polychaete worms.

MATERIALS AND METHODS

Details of the methods used are given in Coyne *et al.* (1994a, 1994b, 1996 and 1997).

The Farm Site

The chosen fish farm site was located off the west coast of County Galway, in Bertraghboy Bay 53°16 N, 9°44 W. The bay is surrounded by a rural community, which has a sparsely dispersed population, and because of the poor quality of the land, agricultural production is low. The fish farm was 1 mile from its land base.

Aqua-Fact International had carried a detailed audit of the farm site out 2 years prior to the current study. Surveying the benthic conditions, including biological and chemical indices, showed a low level of impact under the cages, which became undetectable at a distance of about 10 m from the cages. At the time of this audit, the farm had been in operation for over 10 years. No significant changes in husbandry occurred between then and the beginning of the study.

The unit consisted of two cage blocks, called Blocks 6 and 7, each containing a total of 16 cages, arranged in 2 rows of 8 (Figure 1). Between each cage there was a 2.5m walkway, and a central walkway of 5 m. On occasion, an additional 5 cages could be added, forming a third row, to increase fish holding facilities. From each cage, the nets containing the fish hung 8 m into the water column. The water depth varied from 14 to 17 m and the water column was characterised as ‘neritic / inshore', although periodic intrusions of high salinity, oceanic water were known to occur. The diurnal tides in the bay had maximum amplitude of 5 m. The predominant wind direction in the area was from west to southwest. These physical characteristics ensured good flushing at the site and helped to maintain high oxygen content in the water column.

Aqua-Fact International, took current measurements during a single spring tide cycle, from the north-east corner of cage Block 7 using a surface readout, directional current meter. Measurements were made at 30-minute intervals at the surface, at 5 m depth and off the bottom (18 m).

The wind was light to variable, initially south-easterly and, at the end of the period of observation, south-westerly. The fastest surface water movements, 0.2 m/sec were recorded between 1 and 2 hours after low water, falling to less than 0.1 m/sec at all other times. Mid-water current speeds over 0.1 m/sec were recorded 1 to 2 hours prior to low water, falling to less than 0.05 m/sec for the next 5 hours, to rise again around high water reaching a maximum of 0.15 m/sec. Bottom currents were less than 0.05 m/sec for the 2 hours after low water, but then increased over the next 6.5 hours to a maximum of 0.15 m/sec after high water. The peak surface water movement that occurred after low water was in an easterly direction, but the direction of the mid- and bottom-flows was westerly in the majority of cases.

Sampling methods

Sediment samples were obtained from the points shown in Figure 1. Divers, used a 25 cm long Perspex corer with internal diameter 4.5 cm, to obtain samples for Studies 1 and 2. A long tube attached to a 50 ml syringe was used to remove the

water layer from the core, taking care not to disrupt the sediment layers. The sediments were analysed for OTC as described by Samuelsen *et al*. (1992a).

In the laboratory the remaining seawater was removed from the sediment surface using a 1 ml syringe. For Study 3 and the beginning of Study 4, the length of the coring tube was reduced to 2 cm, to lessen the risk of sediment disruption and for ease of manipulation by the divers. The longer core tube was used again to investigate the depth profile of the sediment at the farm site, towards the latter part of Study 4.

Seawater from sediment cores from Study 2, was removed and retained for analysis in order to determine whether OTC had been transferred into the water from the sediment, during transportation.

For Studies 3 and 4 each salmon sample comprised five specimens collected by net from the cages undergoing chemotherapy. Mussels were collected by divers from weights hanging on ropes flanking either side of the Cage 65 and from the mooring ropes about 1 m from the water surface and 20 m to the east of the block.

All samples, sediment, mussel and salmon, were placed on ice, in an insulated container, immediately after collection. The sediment cores were maintained in an upright position in ice and covered with aluminium foil to reduce the amount of light penetration and to maintain low temperature. Samples invariably reached the laboratory within three hours of sampling and were processed and analysed without delay.

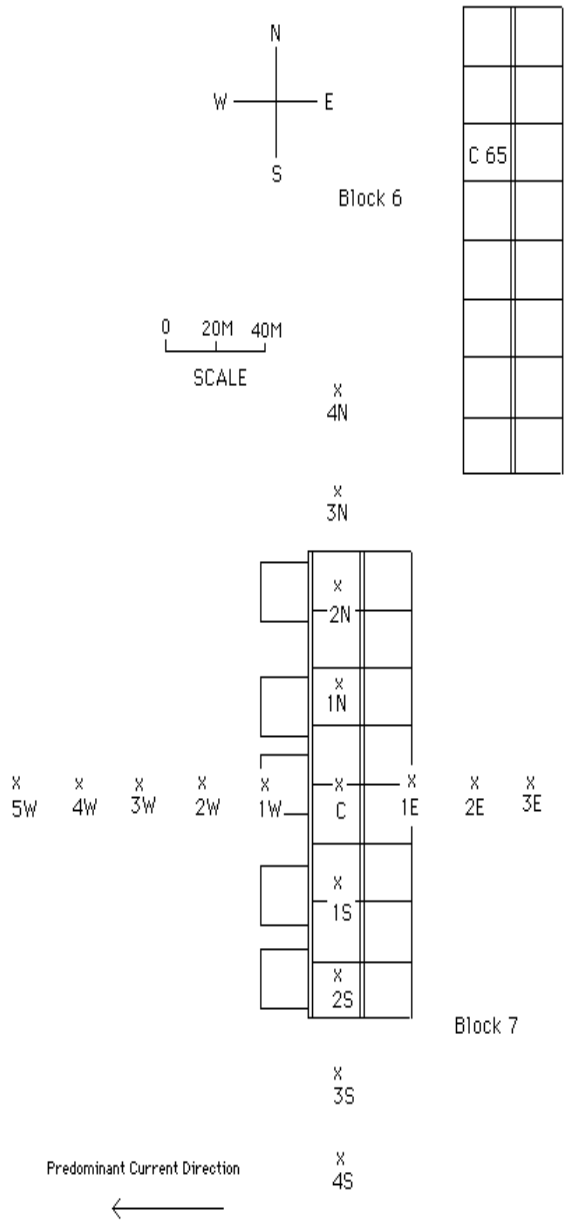


Figure 1. Layout of cage blocks. Sediment sampling sites, shown by 'X' symbols for Block 7, were similarly arranged for Block 6.

Chemical analysis

Details of the laboratory preparation of samples are given in Coyne (1998) and Coyne *et al.* (1994a, 1994b, 1996 and 1997). Isocratic reverse phase HPLC was carried out under the following conditions:

Column: C-18 spherisorb 5 µm 220 x 4.6 mm internal diameter.

Mobile phase: 0.001M Na_2EDTA, 0.05M citric acid, 0.013M trisodium citrate and 0.1M potassium nitrate, as in Knox and Jurand (1979) but rather than dissolution in water-dimethylformamide (82:18, v/v), the buffer was made up in 65% water, 25% dimethylformamide and 10% acetonitrile (v/v/v), pH 3.2.

Detection: UV @ 353 nm

The mobile phase used for HPLC analysis of OTC was a minor modification of the Knox and Jurand (1979) method as formulated by Dr Donlon, Department of Biochemistry, NUI, Galway. The modified method was adopted, as it already proved successful in the analysis of OTC from the effluent of a fresh water fish farm (Smith *et al*., 1994). The extraction buffer for sediment was 0.1M Na_2EDTA-McIlvaine buffer, pH 4.0 (Samuelsen, 1989).

All chemicals used were of analytical grade. OTC was obtained from Sigma Chemical Company, Poole, Dorset. Validation, using spiked samples of sediments, salmon and mussels was also carried out.

Calculations of half-life persistence

Estimation of the half-life of persistence in sediments and mussels was derived from the slope of the plot of Log_{10} OTC concentrations against time. Values were calculated by the curve fit menu of Cricket Graph software version 1.3.2 (Computer Associates, San Jose, CA.)

RESULTS

The sediment

The state of the under-cage sediments comprised of brown to grey muddy sands with total organic carbon from 8.17 to 9.28 mg/g and redox layer at 1 cm during Studies 1 to 3. No outgassing was recorded and only small patches of *Beggiatoa sp.* were seen. Infaunal macrofauna was for the most part restricted to the polychaetes *Capitella capitata* and *Malacoceros fuligenosus.* The latter occurred at a density of approximately 500 m^{-2}. Diversity, evenness and richness (Margalef, 1958) values of 1.30, 0.43 and 1.37 respectively for the three Studies, reflected this faunal impoverishment.

Study 1: Measurement of the horizontal and vertical distribution of the residues resulting from therapy in a single cage block and measurement of the half-life of persistence in the sediment.

Therapy

The salmon were treated with OTC in response to an outbreak of furunculosis. Cage Block 7, with an additional 5 cages to the west, contained a total of 113 t of salmon. The 6 most northerly cages and the 5 additional cages contained a total of 3 t of smolt, while the southern 10 cages contained a total of 110 tonnes of grower salmon.

Until the outbreak of furunculosis, the salmon were fed BP grower. During the outbreak, a medicated feed, surface-coated with antibiotic was used. The dosage was 125 mg OTC per kg body weight for 12 days. The total amount of OTC used was 175 kg.

Residues in sediment

Sediment samples, from the 17 points shown in Figure 1, were collected on day 10 of therapy 2 days prior to and 19, 33 and 71 days after the end of therapy. Table 1 shows the OTC levels according to the depth and time up to Day 33. On Day 71 no residue was detected in any sample. No residues were detected in any of the peripheral points to the north, south or east of the cages. One measurable quantity, 1.4 µg/g was recorded at the nearest point to the west (2 W) during therapy, none were found after therapy had ended.

Residues were detected in the top 2 cm of all undercage points during therapy and at 1W. The sampling point 1W had the highest concentration where OTC reached a depth of between 6-8 cm. At the second sampling, 19 days after treatment had ended, residues in the top 2 cm were greatly reduced, but had increased at greater depths, down to 8 cm. None were detected lower than 10 cm at any time.

Sampling points	*1N*	*C*	*1S*	*2S*	*1E*	*1W*
Distance (m) from C	25	<1	25	50	20	20
Day 10 of 12-day therapy						
0-2 cm	10.0	16.5	13.7	3.8	11.7	18.8
2-4 cm	trace	2.7	<1	<1	<1	3.7
4-6 cm	<1	1.4	<1	<1	<1	1.9
6-8 cm	<1	<1	<1	<1	<1	1.0
Day 19. (19 days after end of therapy.)						
0-2 cm	1.7	4.8	4.3	1.8	2.1	<1
2-4 cm	2.1	4.1	3.7	1.3	1.8	<1
4-6 cm	1.0	5.0	1.8	trace	<1	<1
6-8 cm	1.1	2.0	<1	trace	<1	<1
8-10 cm	trace	trace	<1	<1	<1	<1
Day 33. (33 days after end of therapy.)						
0-2 cm	1.4	1.8	1.6	<1	1.0	<1
2-4 cm	1.0	trace	1.7	<1	<1	<1
4-6 cm	<1	<1	trace	<1	<1	<1
6-8 cm	<1	<1	trace	<1	<1	<1

Table 1. Block 7 OTC concentration µg/g wet weight in undercage sediments in Study 1. Blank spaces indicate no sampling, '0' non-detectable.

Effect of input on residue

The relationship between the quantity of OTC administered in each cage and the accumulation of residues in the underlying sediment is shown in Figure 2. The total quantity administered in the course of the first 10 days of therapy is plotted against the concentration per unit area of sediment surface along the north-south and west-east axis. Close correlations are apparent in both cases, but there is a distinct westward shift in the latter, apparently corresponding to the prevailing direction of sub-surface currents.

Persistence of residues

The half-life of OTC in the sediment was calculated by plotting the mean concentration on the number of days from the end of the 12-day therapy. Residues were detected on day 10 of therapy and post therapy on days 19 and 33 and no traces were detectable at the fourth sampling on Day 71. The half-life of persistence was determined to be 12 days.

Vertical distribution of residues

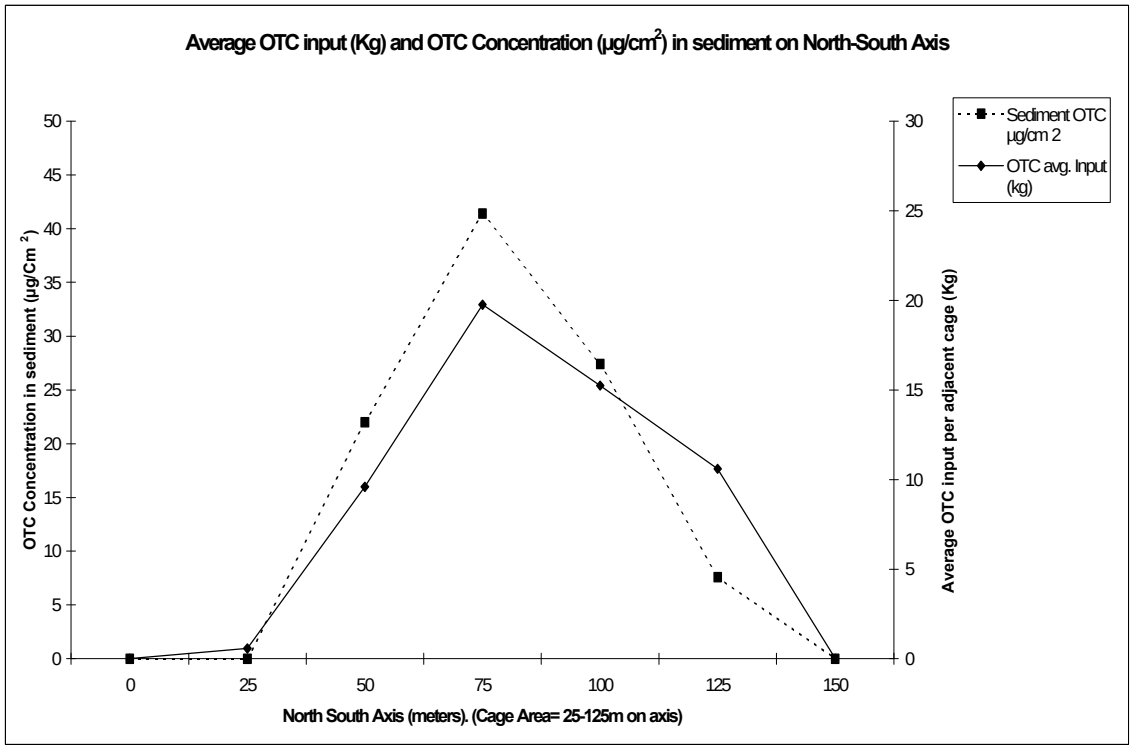
The variations of concentration with depth are shown in Table 1. On Day 10 of the treatment, the upper 2 cm of undercage sediment contained an average of 10.6 µg/g OTC, decreasing rapidly with depth and undetectable below 8 cm. Nineteen days after the end of therapy, the total residue was greatly reduced overall, but had doubled its concentration at depths 2 to 6 cm. On Day 33 it was present only in the upper 4 cm and at little more than trace levels.

Horizontal distribution of residues

On the tenth day of therapy, OTC was present at concentrations between 10 and 20 µg/g in the top 2 cm of sediment in 5 of the 6 undercage sampling points and at 4 µg/g in the sixth point. It was detected at only one of the peripheral points, 2W, which was near (< 20 m) to the cage blocks. The limits of distribution lay within the cage block to the north, less than 20 m from its edge to the east and south and less than 60 m to the west. By Day 19 after the end of therapy, two of the undercage points and all the peripherals proved negative and further OTC reduction in area had taken place at Day 33. On Day 71, no residues could be traced. The maximum extent of the distribution of the residues was less than twice the area of the cage block.

Total quantity of OTC in the sediment

A simple calculation was made, assuming that the core samples were fully representative and that the density of the sediment was 1 g/cc. The total for the entire area of affected sediment came to 1.4 kg, which was approximately 1% of the total input.



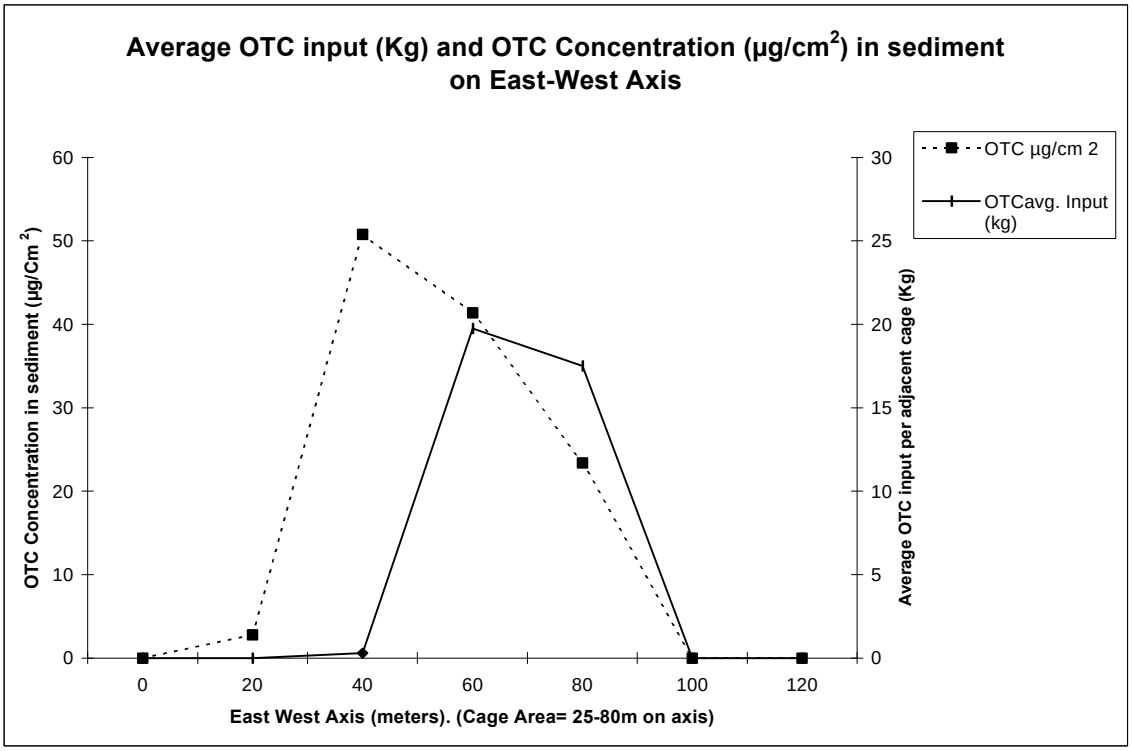


Figure 2. OTC input (total quantity administered in 10 days as kg per cage) to cages above sampling points and concentration in sediments (µg per cm^2) 10 days after beginning of therapy. Points at 25 to 125 m on north-south and 40 to 80 m on east-west axis were under the cages.

Study 2. Comparison of OTC levels in sediment and seawater, resulting from treatment in both blocks of cages, but at different dosages.

Both cage blocks were undergoing OTC therapy concurrently, 20 kg being applied to Block 6 and 175 kg to Block 7. The study comprised sampling of sediment and mussel on the 5th day after therapy ended at Block 6. The seawater above the sediment in the core tube was sampled on return to the laboratory.

Site	*3N*	*2N*	*1N*	*C*	*1S*	*2S*	*3S*	*2W*	*1W*	*1E*	*2E*
Sediment	0	1.3	3.8	4.5	trace	0	0	trace	4.2	0	0
Seawater			0.1	0.2	0		0.1	0	0.3	0	

Table 2. Block 6 OTC concentration µg/g in undercage sediments and in seawater from sediment sample tubes in Study 2. Blank spaces indicate no sampling, '0' non-detectable.

Residue levels in the sediments in all cases were very low, less than 5 µg/g(Table 2). The highest were observed in the centre and towards the north and west. Sampling did not take place far enough to the west to yield a non-detectable value, but a marked decrease along the sampling line was observed, with only traces present at 2W.

Residues were detected in the seawater, in sample tubes. The levels were very low, less than 0.4 µg/g. On average the mussels sampled contained 5.9 µg/g of OTC.

Study 3. Establishing the concentration and half-life of OTC in sediment, the mussel *Mytilus edulis* and salmon in cage Block 6.

In response to an outbreak of furunculosis, a 10-day course of OTC therapy at 125 mg/kg body weight/day was administered to the fish in Block 6. This block contained 144 t of salmon of average weight 890 g distributed uniformly between cages at an average biomass of 9 t. The sampling programme focused on Cage 65, which was stocked with 6.8 t and received 865 g OTC per day. The cages to the north, east and south of Cage 65 sampled received 1,024, 1,120 and 1,696 g per day, respectively. Sampling took place in February.
Sampling dates and results are given in Table 3. Sediment samples were collected immediately before and up to 66 days after the end of therapy. Mussels were collected from two locations: Location 1 directly under Cage 65 at a depth of approximately 10 m, from either side of the cage. Location 2 was 1 m below a surface structure, 20 m to the east of the cage block. Sampling was discontinued there following negative results on each of the first seven occasions. Salmon were taken from Cage 65. Mussels and salmon were collected from immediately before the beginning and up to 37 and 26 days respectively after the end of therapy.

The mean concentration of OTC in the top 2 cm of sediment fell exponentially from 9.9 µg/g on Day 3 to 1.6 µg/g on Day 32 and had disappeared from 4 out of the 5 samples on Day 66. Excluding Day 66, a half-life value of 15 days was calculated from the exponential curve.

The mussels samples in Location 2, in the top 1m of the water column located 20 m to the east of the cage block did not contain oxytetracycline at any stage during or after therapy. The current flow data indicated that they were feeding in water that would have passed through the cage block for some time during each tidal cycle.

	Pre	**During Therapy**					**Post Therapy**											
Day	*-12*	*-9*	*-7*	*-5*	*-4/3*	*-1*	*3*	*5*	*7*	*10*	*12*	*17*	*19*	*25/6*	*30*	*32*	*37*	*66*
Sediment samples																		
1	<1.0						7.6				6.7					1.9		<1.0
2	<1.0						7.8				5.0					2.7		1.6
3	<1.0						8.9				4.0					2.1		<1.0
4	<1.0						14.7				6.0					2.0		<1.0
5	<1.0						10.4				2.5					2.8		<1.0
Mussels – locations 1 and 2																		
1	<0.5	1.5	3.9	10.2	4.7	7.4	4.3	1.6	0.5		<0.5		<0.5	<0.5	<0.5	<0.5		
2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5											
Salmon – individual fish																		
1	<0.5				0.6		trace			2.4		<0.5		<0.5				
2	<0.5				0.8		0.7			2.1		<0.5		<0.5				
3	<0.5				2.7		1.3			2.0		<0.5		<0.5				
4	<0.5				0.9		1.2			1.9		<0.5		<0.5				
5	<0.5				1.7		1.1			2.0		<0.5		<0.5				

Table 3. Block 6 OTC concentration µg/g before, during and after 10 days of therapy (from Day –9 to Day 0) in Study 3. Blank spaces indicate no sampling.

During therapy, concentrations of OTC from 1.5 to 10.2 µg/g were observed in the mussels. Levels fell rapidly as soon as therapy ceased and a half-life of persistence of 2 days was calculated.

In the salmon, on the 7th day of therapy, the mean concentration was 1.5 µg/g. It had fallen on the 3rd day after the end, but had increased to 2.1 µg/g seven days later, equal to 55 degree-days after the end of therapy. From Day 17 (17 days after therapy), 94 degree-days, onwards no traces were observed. Due to the fish-to-fish variation in OTC concentration, the half-life of elimination was not calculated, but in all cases, OTC had become untraceable in the tissues between 10 and 17 days after therapy.

Study 4. Determining the concentration and half-life of OTC in sediment, salmon muscle and mussel tissue after prolonged treatment.

The medicated feed administered contained 3.2 % w/w OTC that had been surface coated onto the pellets by the feed manufacturer. The dosage of OTC was 125 mg/kg fish weight/day. Immediately after the start of treatment, the feeding response of the fish fell rapidly to approximately 50 % of normal values and only recovered slowly. Consequently, treatment was continued for 24 days. A total of 529 kg or an average of 1.4 kg per cage per day was administered.

Sediment samples were taken according to the cross shaped sampling regime shown in Figure 1. Sampling took place on 12 occasions, from Days 3 to 89 after the end of therapy. Five fish samples were collected from Cage 74 on the same sampling days up to Day 62. Mussel were collected, from mooring ropes approximately 1 m above the sediment under Cage 74, 2 days before the end of therapy and on the same days that the sediment were sampled to Day 38.

The pattern of concentrations in the sediment, shown in Table 4, was similar to that observed in the other studies. In general, the highest values were recorded under the cages in the samples taken soon after the end of therapy. OTC concentrations decreased with distance from the cages and with time. OTC remained detectable up to 90 days at 4 of the 5 undercage points. It was found to have fallen to very low levels after 62 days and was only found at one point after 165 days. High concentrations of 7 and 12 µg/g were observed at 1W on Days 10 and 17 after therapy, following negative samples both before and after. There were no indications of “hotspots” that could be attributable to sampling an intact medicated feed pellet in the sediment cores. OTC concentrations were consistently low (< 19 µg/g) which could suggest some reprocessing of medicated feed by benthic macrofauna.

Vertical distributions of OTC were measured on Days 90 and 189. By Day 90, only 2 of the 6 undercage points were positive in the upper 2 cm and the highest concentration was 1.0 µg/g at 1S. Traces were detected at 2S and these extended downwards to 6 cm. At greater depths, no residues could be detected. On Day 189 only one point, 1E gave a positive result. A concentration of 2.0 µg/g was observed at depth 2 – 4 cm but OTC was absent from all depths at the other 4 points.

A month after the end of therapy, OTC concentrations in the top 2 cm of undercage sediment averaged 1.2 µg/g in Study 1 and 2.3 µg/g in Studies 3 and 4. After 2 months OTC concentrations were 0.3 µg/g in Study 3 but 1.5 µg/g in Study 4. In Study 4, the OTC therapy had been followed by a period of over-feeding during a subsequent outbreak of pancreas disease. This had led to an increase in accumulation of feed pellets on the sediment and consequent de-oxygenation of the sediments with a reduction in the density of polychaete worms. It is possible that these events led to greater persistence of the OTC residues. Only two traces were found at the peripheral sampling points after Day 45.

During Therapy		**Post Therapy**											
Day	*-2*	*3*	*6*	*10*	*17*	*24*	*30*	*38*	*45*	*62*	*90*	*165*	*189*
Sediment N to S													
4N		<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
3N		trace	trace	<1.0	trace	<1.0	<1.0	<1.0	trace	<1.0	<1.0	<1.0	<1.0
2N		trace	trace	2.6	2.4	trace	2.3	2.3	0	<1.0	<1.0	<1.0	<1.0
1N		8.9	3.3	2.2	3.4	trace	4.7	4.7	2.7	4.1	trace	<1.0	<1.0
C		8.7	1.8	4.7	5.4	3.7	0	trace	1.0	trace	1.0	<1.0	<1.0
1S		2.1	3.8	1.9	2.1	1.0	3.6	trace	3.6	1.7	1.0	1.0	0
2S		5.6	2.2	2.6	2.1	1.0	0	1.1	<1.0	<1.0	trace	<1.0	<1.0
3S		<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0				
4S		<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0				
Sediment W to E													
3, 4 and 5 W		<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2W		1.3	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1W		<1.0	<1.0	12.2	7.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1E		1.3	trace	2.9	2.2	0	trace	trace	trace	1.1	trace	<1.0	<1.0
2 and 3 E		<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Salmon													
1		3.1	<0.5	1.9	trace	trace	<0.5	<0.5	<0.5	<0.5			
2		4.4	<0.5	2.4	<0.5	trace	0.5	<0.5	<0.5	<0.5			
3		4.1	<0.5	1.8	0.5	trace	0.5	<0.5	<0.5	<0.5			
4		5	1.1	1.3	trace	<0.5	<0.5	<0.5	<0.5	<0.5			
5		4.2	<0.5	1.4	0.5	0	0	<0.5	<0.5	<0.5			
Mussels													
	1.6	1.1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5					

Table 4. OTC concentration µg/g, during (ending on Day 0) and after 24 days of therapy in Study 4. Blank spaces indicate no sampling.

From the data in Table 4 a mean value of 4.2 7 µg/g in salmon was calculated for Day 3. On Day 10 this had fallen to 1.7 µg/g. On days 17 to 30, traces were present in decreasing numbers of fish and no OTC was detected from Day 38 onwards. Variation in concentration between fish precluded calculation of a value for the half-life of persistence.
Low levels of OTC were observed in mussel during therapy and on Day 3, after which all samples were negative.

Sediment concentration relative to input

The ratio of the concentration of OTC in the top 2 cm of undercage sediment to the input of OTC was used to compare the relationship of input to residue between the four studies. Table 5 shows close agreement between the first three, but a ratio an order of magnitude lower in the case of Study 4. The therapy in Study 4 was different in both the duration and the total input being at least double that of the others.

Study	*Duration (days)*	*Day no. (during and after(+) therapy)*	*Input*	*Concentration*	*Concentration /input*
1	12	10	8.3	10.8	1.3
2	12	+5	1.3	2.1	1.6
3	10	+3	8.6	9.9	1.2
4	24	+3	33.0	4.6	0.14

Table 5. Duration of therapy (days), day number (days during and after end of therapy), mean inputs of OTC (kg/cage) and concentration in upper 2 cm of sediment (µg/g) and concentration as a function of input.

Predictive modelling

Sediments

Data from Cage Block 7 in Study 1, on current flow from Aqua-Fact International and deposition rates of 0.12 and 0.04 m/second for food and faeces, respectively given by Gowen *et al*. (1989) were used to model the area of sediment that would be affected. The model was used first to predict the deposition resulting from a point at the NE corner of the cage block. This was then generalised to account for the entire cage block. The results are shown in Figure 3.

The model used takes no account of the effects of microturbulence, haloclines or thermoclines, all of which may exert significant effects on the behaviour of very light faecal particles. The model predicts that uneaten feed will be deposited in an area extending 30 m to the west of the cages, approximately 8 m to the north and south and less than 2 m to the east. Similar figures for the area predicted to be subject to deposition of faeces would be 60 m to the west, 30 - 35 to the north and south, and less than 10 m to the east. The dominant westerly movement of solid material from the cages, predicted from the data on current flow, was supported by the observations of divers and previous photographic surveys of the sediments under these cages.

In Study 1, the area of sediment positive for OTC, after 10 days of therapy, was found to correlate well with the area predicted to be subject to feed deposition. Only one sample point within this area proved negative for OTC. This was the most northerly of the under-cage points. The cages had been the most lightly stocked and therefore treated with the smallest quantity. OTC was not detected in samples taken from the Area 2 in which faecal, but no feed, deposition took place.

Estimate of concentration of OTC in the water column

The following estimate is based on the antibiotic therapeutic input data from farm records and the volume of water occupied by the holding capacity of a farm cage. Assuming that the total amount of oxytetracycline administered to Cage 65 in Study 3 remained dissolved in the water contained within in the farm cage, the resultant concentration can be estimated. The water volume was 15 x 15 x 10 m = 2.25×10^9 ml). Over the ten days of therapy 8,650 g were administered, giving a concentration of 3.8 µg/ml.

However, these calculations assume that all oxytetracycline administered is dissolved in the water and there is no water exchange. Assuming a total water exchange once an hour, the concentration would be 0.016 µg/ml and far below the detectable limit.

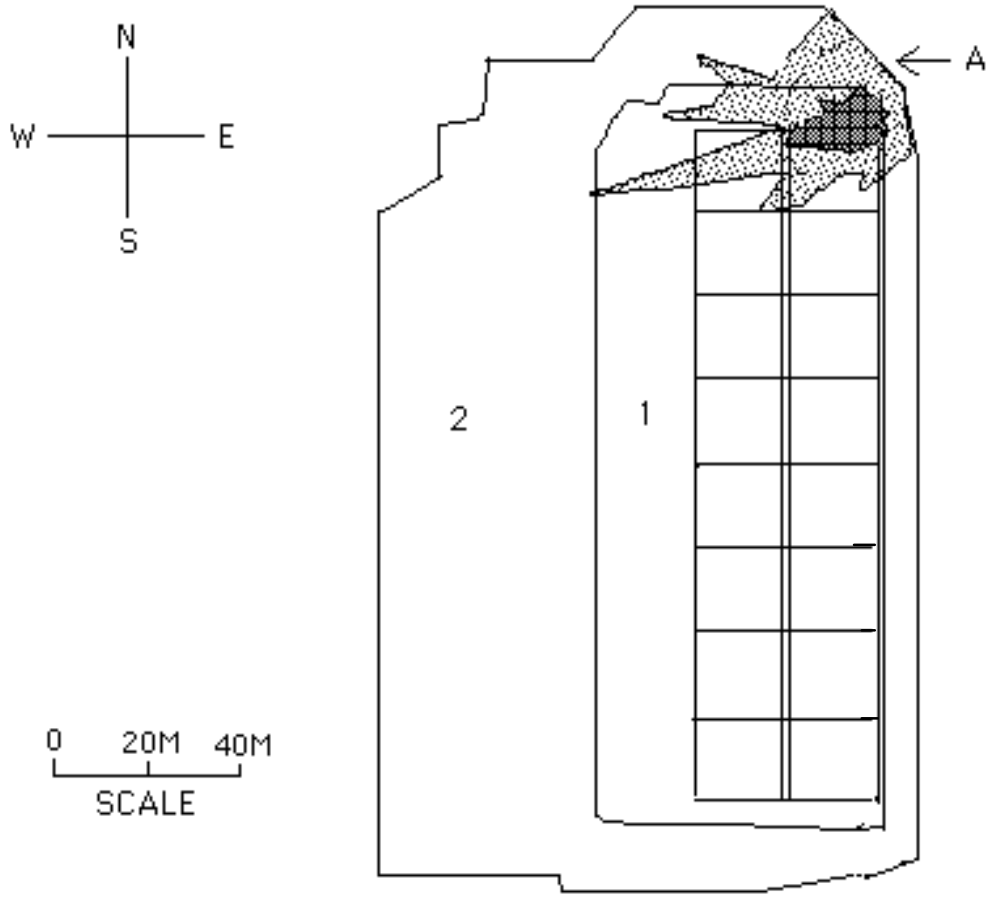


Figure 3. Computer model prediction of the area of sediment subject to deposition. A: effects from a point source; dark hatching = feed, light hatching = faecal deposition. Area 1 = feed, Area 2 = faecal from the whole cage block.

DISCUSSION

The use of OTC as an effective treatment for furunculosis has been established since the 1960s. Its acute oral toxicity is very low, with LD_{50} values > 4000 mg/kg, very much higher than the effective dose of 50 to 100 mg/kg (Bjørklund and Bylund, 1991). Therefore, OTC has been very widely used in Irish salmon farms where furunculosis has been prevalent. Its use is permitted by the relevant EU veterinary medicine legislation as set out in Council Regulation 2377/90.

Residues in sediments

Concentrations

The work of Jacobsen and Berglind (1988), Bjørklund *et al*. (1990), Samuelsen *et al*. (1992a) and Capone *et al*. (1996) had shown that residues of OTC were observed in sediments in the vicinity of marine cage farms and in filter-feeding molluscs such as the mussel (*Mytilus edulis*). The sediment concentration ranged from 0.1 to 4.9 µg/g in 12 sets of observations from Norway, Finland and North America. These were somewhat lower than those observed in the present work which had a maximum of 18.8 µg/g. The value of 475 µg/g reported by Samuelsen *et al*. (1992a) was so far out of line with all the others that it suggests the operation of some exceptional factor.

Input quantity and residue concentration

The ratio of concentration in the sediment in µg/g to input of OTC in kg/cage was used by Smith (1996) to compare the results of various studies. In 8 sets of observations by Bjørklund *et al.*1990, 1991 and Capone *et al.* (1996), the ratios observed ranged from 0.08 to 1.0 µg/g/ kg/cage . These are similar to the range, 0.14 to 1.6 µg/g/kg/cage in the 4 studies in the present work. Based on the residue concentrations, simple calculations were made of the total quantity of OTC in the sediment and these were related to the known total input to the cages. Values for total quantity extrapolated from 7 observations by Bjørkland *et al.* (1990) and Capone *et al.* (1996) ranged from 0.3 to 8.5 % of total input. Values in the 4 studies in the present work were similar, ranging from 1 to 5%.

Persistence

Studies that reported prolonged oxytetracycline half-lives, Samuelsen *et al*. (1992a) and Bjørklund *et al*. (1990), shared a common thread of poor sediment quality, probably associated with overfeeding and consequential build up of an organic rich layer on the sediment surface. Samuelsen *et al*. reported that the sediment they collected was anoxic up to 50 cm deep, was black and smelled of H_2S. At these sites the half-lives at different cages ranged from 89 to 142 days. Bjørklund *et al*. reported a half-life of 419 days at a site where the depth of organic detritus under the cages was 10 - 30 cm. A half-life of 12 days was calculated in Study 1 of the present work. All traces of residue had disappeared by Day 33 in Study 1. Longer persistence, greater than 45 days was observed at one point in three in Studies 3 and 4. At the end of the longest post-therapy period measured, 189 days, no traces could be detected. As observed by the other authors, persistence was longer where over-feeding had led to an accumulation of organic debris above the sediment.

Vertical distribution

Samuelsen *et al*. (1992a) reported that soon after the completion of therapy, the maximum depth of OTC burial in the sediment was 10 cm. Subsequently, OTC disappeared from the upper 2 cm more rapidly than from the lower levels. These observations are in agreement with the data obtained in a slightly more artificial system by Nygaard *et al.* (1992). Similar observations were made by Hektoen *et al*. (1995). Samuelsen *et al*. suggested that a gradual covering of the oxytetracycline-containing layer with additional faecal and food residues results in reduced contact with the overlying seawater and a decrease in the speed of its removal.

The present results agree in general with these observations. Highest concentrations were observed in the upper 2 cm during the therapy stage, with traces as far down as 8 cm. After an interval of 19 days, the concentration in the upper layer had fallen from 12.4 to 2.4 µg/g. But the mean at depth 2 to 4 cm had increased to 2.2 µg/g, almost equal to that in the upper layer. Concentrations then decreased with increasing depth and could be detected down to 8- 10 cm. On Day 33, the mean concentration in the upper layer was 1.0 7 µg/g, double that at depth 2 to 4 cm. The general pattern therefore was of a steady decrease in total concentrations with time but some evidence of downward movement within the sediment column.

Horizontal distribution

The area of sediment containing measurable residues was examined by Capone *et al*. (1996). They found OTC to be localised, detectable only in the direction of the prevailing currents. At the farm that used the smallest quantity (0.3 kg), traces were found only beneath the cages. Two other farms used larger quantities, 9.5 and 186 kg. Residues were detected at 30 m distance, but no residues were detected at 100 m. In the present study residues were detected at a greater distance, up to 125 m but absent at 150 m. Differences in local current speeds could account for the difference, but in both cases horizontal dispersal of OTC in the sediments was insignificant except in the direction of the prevailing current. This affected area was less than that of a cage block.

Residues in other organisms

Salmon

In the farm study by Bjørklund and Bylund (1990) the maximum OTC concentration achieved in rainbow trout was 1.5 µg/g. Namdari *et al*. (1998) recorded maximum values of 0.78 µg/g for chinook salmon held at 9ºC and 1.04 µg/g at 12ºC. Atlantic salmon received the same dose as chinook and the maximum concentration was 1.21 µg/g at 7 ºC. A similar result, 1.36 µg/g was observed in laboratory studies of chinook salmon (Aoyama *et al*. 1991). These were very similar to the 1.1 µg/g observed three days after the 10 days of therapy in Study 3.

Higher values were observed when therapy extended for longer periods. Laboratory studies were designed by Namdari *et al*. (1998) to establish a maximal withdrawal time in chinook and coho salmon by using prolonged therapies of 21 days for chinook salmon and 42 days for coho. These resulted in concentrations up to 5.6 µg/g for the chinook and 2.0 µg/g for the coho. In Study 4, the concentration in salmon muscle was 4.2 µg/g.

In the case of the short treatment in Study 3, traces of OTC were detected on Day 10 in salmon muscle but not on Day 17. However, higher muscle concentrations and longer persistence, up to Day 30 was observed in Study 4 following a long therapeutic treatment.

Mussel (Mytilus edulis)
Samuelsen *et al*. (1992b) investigated oxolinic acid levels in mussels in the vicinity of a commercial farm and reported a mean antibiotic concentration of 0.65 µg/g (n=5), reaching non detectable levels on Day 7 post therapy. Møster (1986) reported OTC concentrations of 7 µg/g in mussels and a half-life of 8.5 days, after therapy in a commercial farm, using a biological method of analysis. In Studies 2, 3 and 4, OTC concentrations of 1.6 µg/g to 5.9 µg/g were observed. The decline of OTC concentration in the mussel tissue was rapid and the half-life of persistence was 2 days.
Other organisms
It seems likely that the worms, plentiful in the sediments beneath the cages studied in this work, ingested the sediment containing oxytetracycline and were important in bringing it to the lower layers of the sediment. However, the one study in which attempts were made to collect sufficient polychaete worms for OTC analysis coincided with a period of over-feeding at the farm and a consequent reduction in worm numbers.
Samuelsen *et al*. (1992b) and Ervik *et al*. (1994) provided evidence to suggest that the primary source of drug concentrations in wild fish was the consumption of medicated feed. Smith *et al*. (1997) reported observations which implicated green crab *Carcinus maenas* and possibly other macrofauna, as important scavengers in the removal of feed pellets from the sediment at the farm investigated in this work. The following species were also considered likely to contribute to the removal of food residues: the starfish *Asterias rubens* and *Marthasterias glacialis*, the blenny *Lipophrys pholis* and dragonet *Calionymus lyra*. Capone et al. (1996) reported that approximately half the red rock crab *Cancer productus* collected during therapy and 12 days after its end contained OTC in meat at concentrations up to 3.8 µg/g. This persisted for at least 2 weeks and traces were detected in two crabs collected 41 and 75 days after therapy. In contrast to the red rock crab the Dungeness crab, *Cancer magister* had no more than traces at any time.
In fresh water, Bjørklund *et al*. (1990) found residues of oxytetracycline in wild bleak *Alburnus alburnus* collected on the last day of medication, up to 1.3 µg/g. A week after therapy, OTC levels were reduced to traces. On another farm, roach *Rutilus rutilus* contained traces up to 13 days while bleak were positive for only 1 day after the end of therapy. Overall the concentration of oxytetracycline in these wild fish was low.

Total budget
In Study 1, it was estimated that the OTC in the sediment, 10 days after a 12-day therapy, represented 1.0 % of the total input. Study 3 indicated that the contribution of mussels as a reservoir would be negligible, being less than 0.1% . In Study 4, use of the predictive model indicated that the standing crop (179 t salmon) contained, shortly after the end of therapy, approximately 0.2 % of the OTC administered. Thus, the total quantity accounted for in these compartments was 1.3 % of the input. In the absence of any other obvious carriers, it appears that approximately 98.7 % has been "lost".

CONCLUSIONS

In the case of the salmon farm in Bertraghboy Bay investigated in this work, there are clear indications that sediment deposition is not the fate of the greater part of the OTC used in therapy. However, analysis of the data obtained in this work suggests that the greatest concentrations in the environment, and therefore the greatest biological impacts, occur in the sediment. The area of sediment affected is little more than that situated immediately below the fish cages. Moreover, all but traces of these residues disappear within one month after the end of therapy.

Estimates of the concentration that may occur in the water column would suggest that even if all the OTC were to remain in this compartment it is unlikely that it would ever reach biologically significant concentrations.

Estimates of both the concentrations and persistence of OTC in the sediments under farm cages vary widely. From the work presented here and the analysis of the published work of other investigators it is possible to identify the key factors that affect these two parameters. It is clear that high concentrations have been recorded only at sites where there was a significant over-presentation of feed. Equally, long term persistence occurs at sites where significant organic deposition has resulted in anoxic sediments with drastically reduced infaunal populations.

These observations suggest that a possible adverse impact of OTC in sediments will occur only at sites where other aspects of husbandry lead to significant deterioration of sediment quality. Routine monitoring of sediment quality, by techniques such as redox measurements, would allow logistically simple and cost-effective identification of farm sites where antimicrobial use might cause an adverse environmental impact.

The overall conclusion from the work is that OTC residues do not persist or disperse so widely that their use in the treatment of cage-reared salmon offers negligible risk to the marine environment, or to human health.

REFERENCES

Alderman, D. J. 1999. Requirements for the approval of veterinary therapeutics or growth enhancers in fish production: European Union. Chapter 3 in D. J.Smith, W. H. Gingerich and M. G. Beconi-Barker (eds.). *Xenobiotics in Fish*. New York, Kluwer/Plenum Press.

Anon. 1997a. *Aquaculture*. WWW.BIM.ie/97 highlights/4.htm.

Anon. 1997b. *Note for guidance on the establishment of maximum residue limits for Salmonidae and other fin fish.* EMEA/CVMP/153b/97-Final.

Anon. 1997c. Towards safe and effective use of chemicals in coastal aquaculture. *GESAMP Report* 65 : 40p.

Aoyama, G. R, M. K. McErlane, and H. Erber, 1991. High performance liquid chromatographic analysis of OTC in chinook salmon following administration of medicated feed. *Journal of Chromatography* 588, 181 - 186.

Austin, B. and Austin, D. A., 1993. *Bacterial Fish Pathogens: disease in farmed and wild fish*. Ellis Horwood Series in Aquaculture and Fisheries Support, London.

Bjørklund, H. and G. Bylund, 1990. Temperature-related absorption and excretion of OTC in rainbow trout (*Salmo gairdneri* R.). *Aquaculture*, 84, 363 - 372.

Bjørklund, H., J. Bondestam and G. Bylund, 1990. Residues of OTC in wild fish and sediment from fish farms. *Aquaculture*, 86, 359 - 367.

Bjørklund, H. and G. Bylund, 1991. Comparative pharmacokinetics and bioavailability of oxolinic acid and oxytetracycline in rainbow trout (*Oncorhynchus mykiss*). *Xenobiotica*, 21, 1511 - 1520.

Bjørklund, H., C. M. I. Rœberg and G. Bylund, 1991. Residues of oxolinic acid and OTC in fish and sediment from fish farms. *Aquaculture* 97, 85 - 96.

Capone, D. G. D. P. Weston, V. Miller and C. Shoemaker., 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. *Aquaculture* 145, 55 - 75.

Coyne, R., M. Hiney, B. O'Connor, J. Kerry, D. Cazabon and P. Smith, 1994(a). Concentration and persistence of oxytetracycline in sediments under a marine salmon farm. *Aquaculture* 123, 31-42.

Coyne, R., M. Hiney and P. Smith, 1994(b). Evidence associating overfeeding on a salmon farm with a prolonged half-life of oxytetracycline in under cage sediments. *Bulletin of European Association of Fish Pathologists*. 14, 207-210.

Coyne, R., M. Hiney and P. Smith, 1996. Quantitation of sediment by oxytetracycline under a marine fish farm. *in* N. Haagsma and A. Ruiter, (eds). Veldhoven, The Netherlands : 357 - 361.

Coyne, R., M. Hiney and P. Smith, 1997. Transient persistence of oxytetracycline in blue mussels (*Mytilus edulis*) following its therapeutic use at a marine Atlantic salmon farm. *Aquaculture* 149, 175 - 181.

Coyne, R., 1998. The use of HPLC to investigate the fate of oxytetracycline in the marine environment of a fish farm. PhD thesis, National University of Ireland, Galway.

Ervik, A., B. Thorsen, V. Eriksen, B. T. Lunestad and O. B. Samuelsen, 1994. Impact of administering antibacterial agents on wild fish and blue mussels (Mytilus edulis) in the vicinity of fish farms. Diseases of Aquatic Organisms. 18, 45 - 51.

Gowen, R. J., N. B. Bradbury and J. R. Brown, 1989. The use of simple models of assessing two of the interactions between fish farming and the marine environment. in: N. dePauw, E. Jaspe, H. Ackefors and N. Wilkins (eds). *Aquaculture - A Biotechnology in Progress*. Bredene, European Aquaculture Society. pp 10711-1080.

Hektoen, H., J. Berge, V. Hormazabal and M. Yndestad, 1995. Persistence of antibacterial agents in marine sediments. *Aquaculture* 133, 175 - 184.

Hiney , M., 1998. Furunculosis. in Smith, P. (Ed). *Fish farming and Our Environment*, The Ardilaun House Hotel, Taylor's Hill, Galway.

Jacobsen, P. and Berglind, L., 1988. Persistence of OTC in sediments from fish farms. *Aquaculture* 70, 365 - 370.

Knox, J. and J. Jurand. 1979. Mechanism of reversed-phase separation of tetracyclines by high performance liquid chromatography. *Journal of Chromatography* 186, 763 - 782.

Lunestad, B. T., 1992. *Environmental effects of antimicrobial agents used in aquaculture*. PhD thesis, University of Bergen.

Møster, G., 1986. *Brul av antibiotika i fiskeoppdrett (Use of antibiotics in Norwegian aquaculture).* Sogn og Fjordane Distriktshogskole, 5800 Sogndal, Norway. pp 58.

Margalef, D. R., 1958. Information theory in ecology. *Gen. Sys*., 3 : p. 36 - 71.

Namdari, R., S. Abedini, L. Albright and F. C. Law, 1998. Tissue distribution and elimination of OTC in sea-pen cultured chinook salmon (*Oncorhynchus tshawytscha),* and Atlantic salmon (*Salmo salar),* following medicated-feed treatment. *Journal of Applied Aquaculture*, 81 (8), 39 - 51.

Nygaard, K.; B. T., Lunestad, H. Hektoen, J. A. Berge, and V. Hormazabal, 1992. Resistance to oxytetracycline, oxolinic acid and furazolidone in bacteria from marine sediments. *Aquaculture,* 104 : p. 31-36.

O'Connor, B., 1998. The impact of organic wastes from marine farms on water and sediment quality in Irish coastal waters. In Smith, P. (ed.). *Fish Farming and our Environment,* The Ardilaun House Hotel, Galway, Ireland.

Samuelsen, O. B., 1989. Degradation of oxytetracycline in seawater at two different light intensities, and the persistence of oxytetracycline in the sediment from a fish farm. *Aquaculture,* 83 : 7 - 16.

Samuelsen, O. B., V. Torsvik and A. Ervik. 1992(a). Long range changes in OTC concentration and bacterial resistance towards OTC in a fish farm sediment after medication. *The Science of the Total Environment* 114, 25 - 36.

Samuelsen, O.B., B. T. Lunestad, B. Husevœg, T. Holleland and A. Ervik, 1992(b). Residues of oxolinic acid in wild fauna following medication in fish farms. *Diseases of Aquatic Organisms* 12, 111 - 119.

Smith, P., 1992. Antibiotics and the alternatives. in N. de Pauw and J. Joyce (eds): *Aquaculture and the Environment.* European Aquaculture Society. pp 223 - 234.

Smith, P., 1996. Is sediment deposition the dominant fate of OTC used in marine salmonid farms ? A review of available evidence. *Aquaculture* 146, 157 - 169.

Smith, P., J. Donlon, R. Coyne and J. D. Cazabon, 1994. Fate of oxytetracycline in a fresh water fish farm: influence of effluent treatment systems. *Aquaculture* 120, 319 - 325.

Smith, P., G. Edwards, B. O'Connor, M. Costello, and J. Costello, 1997. Photographic evidence of the importance of macrofauna in the removal of feed pellets from the sediment under marine salmon farms. *Bulletin of European Association of Fish Pathology* 17, (1), 23 - 25.

Suter II, G. W., 1993. *Ecological Risk Assessment*. London, Lewis Publishers.

Wheatley, S. D., 1994. *Epidemiological investigations of Atlantic salmon production using a computerised data base system*. PhD thesis, Queens University, Belfast.